OBSERVATION OF TWO PHOTOREACTIONS IN PHOTOSYNTHESIS

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A considerable body of indirect evidence obtained in the last few years indicates that more than one pigment must be activated by light for efficient photosynthesis to occur. It has been postulated that different photochemical reactions are carried out by the various pigments (Emerson, et al., 1957; French & Myers, 1960). We have now found that the formation of different photoproducts by different wave lengths of photosynthetically active light can be directly observed by electron paramagnetic resonance (EPR) spectroscopy.

The presence of EPR signals in illuminated photosynthetic organisms has been known for a number of years (Commoner, et al., 1957; Tollin, et al., 1958; Allen, et al., 1960; Treharne, et al., 1960). Most workers have reported a single banded signal with a half width of around 15 gauss in green plants, although Commoner and associates (1957) have reported two signals, one with fine structure, on illumination of spinach chloroplasts. It has also been inferred that the signal is due to the presence of more than one radical because of the complex decay curves obtained on darkening the photosynthetic material (Commoner, et al., 1957; Tollin, et al., 1958; Allen, et al., 1960). We have now been able to resolve the signal obtained from Chlorella pyrenoidosa into two signals differing in line width, relaxation time, and half life. Moreover, it has been shown that one of these signals is induced by longer wave lengths of light, absorbed by chlorophyll a, and the other by shorter wave lengths of light, absorbed by chlorophyll b. The over-all signal resulting from illumination with white light, as well as that in the dark, resembles those observed by Commoner, et al.

The experiments were carried out with 3-4 day old cultures of Chlorella pyrenoidosa illuminated with high intensity monochromatic light. Light from a 900 watt projection lamp was passed through a suitable optical system to collimate it, and thence through interference filters supplemented with appropriate blocking filters to obtain selected wave lengths. EPR measurements were made with a standard Varian Associates EPR spectrometer, equipped with a six inch magnet, a slotted cavity to permit illumination of the cell suspensions, and a flat quartz cell to permit sensitive measurements to be made on cell suspensions in good physiological condition.

Typical experimental results are shown in Figure 1. On illumination with long wave length red light a sharp signal is produced, as shown in (a). When the light is turned off, this signal decays with a half life of 50-60 milliseconds. On illuminating with shorter wave length light, a broader signal is induced, as shown in (b). This signal persists for a long time after darkening, as shown in (c). Its decay is affected by inhibitors and by aeration of the cell suspension, hence its disappearance is probably due to reactions with other cellular constituents. As the microwave power is increased, the sharp signal saturates, while the broader one does not, indicating different relaxation times. The minimal signal remaining after a long dark period is shown in (d).

Because of the overlapping absorption bands of chlorophylls a and b, the wave lengths chosen for this demonstration (710 and 540 mμ) were those on the extremes of the bands in the action spectrum for induction of EPR signals by these pigments. In regions where the absorption bands of the two chlorophylls overlap, a mixture of the two EPR signals is obtained. The action spectrum for induction of the over-all EPR signal in Chlorella shows two peaks, one at 695 mμ, indicating that chlorophyll a-695 is the active form of the pigment in the radical forming reaction, and another in the region of chlorophyll b absorption. Both peaks are rather broad, with long tails.

Confirmation that one of the signals is due to a reaction mediated by

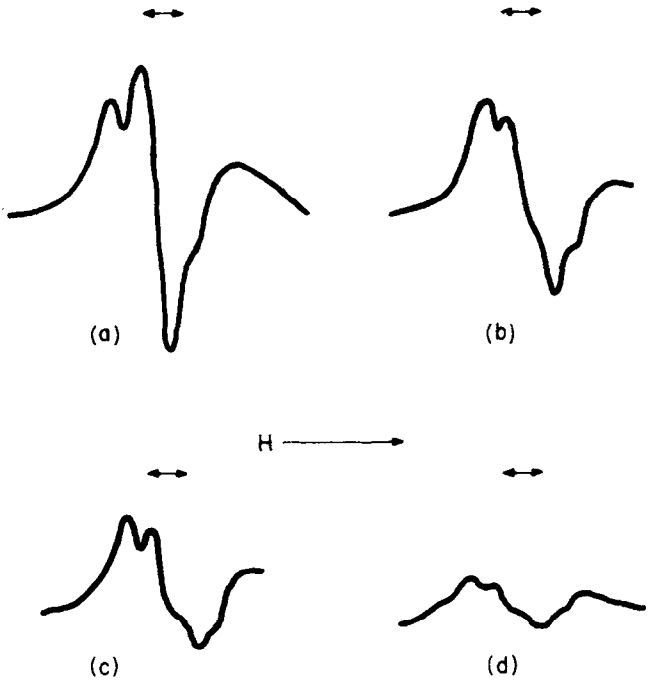

Figure 1. EPR signals observed in suspensions of Chlorella pyrenoidosa. (a) Illuminated with 710 mμ light; (b) illuminated with 540 mμ light; (c) 3-4 minutes after illumination as in (b); (d) after 30 minutes in darkness. ⟷ indicates 11 gauss. Modulation amplitude 1.2 gauss.

chlorophyll a and the other to a reaction involving chlorophyll b was obtained from experiments with a UV induced Chlorella mutant that lacks chlorophyll b (Allen, 1958). On illumination of this organism, only the sharp, rapidly decaying signal ascribed to chlorophyll a was observed.

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